No-pain Removal of HPV Lesions in Oral Cavity

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Human Papilloma Virus (HPV) infection: verruca vulgaris (VV), squamous cell papilloma (SP), condyloma acuminatum (CA), and multifocal epithelial hyperplasia (MFEH), all of them are a benign hyperplastic exophytic proliferation of the oral epithelium, caused by different HPV genotypes. Subtypes 6 and 11, with a low-oncogenic risk, are the most commonly found and cause CA in both the oral cavity and in the anogenital region. Labial mucosa, soft palate and lingual frenum are the most common locations of CA and koilocytes can be observed in histopathologic sections. All HPV-related oral lesions present clinical similarities, and therefore, a biopsy is necessary for a precise diagnosis.

Keywords: oral condyloma ; Human Papilloma Virus ; Ketorolac ; pain ; inflammation ; sodium alginate ; hyaluronic acid

1. Introduction

Although CA is considered a benign lesion, clinical infections with the high-risk genotypes 16 and 18 have been found to cause oral and genital CA and have been associated with malignant lesions [1]. Spontaneous remission of oral CA is possible [2], but if this is not the case, there are different treatments to eliminate it. Surgical therapy seems to be the preferred treatment over Trichloroacetic acid (TCA), cryotherapy, and CO_2 laser because these methods often induce artifactual changes that compromise the diagnostic capabilities of the pathologist [3]. Surgical or ablation treatments have the advantage in that the lesion (s) are removed in a single session and, typically, are quick interventions. However, they are procedures that generally require anesthesia and the control of pain and inflammation. The possibility of recurrences should not be ruled out. In addition to lesions caused by HPV, there are a variety of conditions and diseases of the oral cavity requiring surgical, ablative, or extractive interventions that involve mild to severe pain and inflammation, such as certain tumors of the oral mucosa, temporomandibular joint pathologies, facial trauma, and which could require dental interventions, etc. Pain management in minor surgical and ablative treatments does not always attract the attention it deserves, even though it is crucial for patient satisfaction in that they feel well and are mightily encouraged to follow treatment adherence. Surgery and ablative techniques usually require prior local anesthesia, and the postoperative pain and inflammation should also be controlled. For pain management of these processes, analgesics, anesthetics, and anti-inflammatory drugs can be combined in various regimens.

Ketorolac is a non-steroidal anti-inflammatory drug (NSAID) with a potent analgesic effect and a moderate anti-inflammatory action. It is indicated to treat moderate to severe pain. The analgesic ketorolac potency has been equated to that of several opioids [4] without presenting the problems associated with these drugs, such as tolerance or sedation [5]. Its use both pre [6] and postoperatively [7] has been analyzed, showing successful results. Ketorolac is marketed as tromethamine salt and can be administered orally, intramuscularly, intravenously, and by nasal or ophthalmic processes. Several authors have studied the analgesic safety and efficacy of ketorolac tromethamine (KT) after its topical application in different mucoadhesive formulations on the oral mucosa [8][9]. The results were satisfactory and promising. Therefore, we are able to propose its use during the removal of oral condyloma.

Mucoadhesive topical formulations have advantages over the most common routes, such as a simple and painless application and a better bioavailability of the active ingredient, allowing formulations with lower doses and inducing fewer side effects [10]. However, when formulating drugs intended to be applied to the oral mucosa, certain aspects need to be considered and may limit the success of our formula. One of them is the biology and histology of the mucosa. The lining of the oral cavity includes the buccal (cheeks), sublingual, gingival, palatal, and labial mucosa. These are made up of closely compacted epithelial cells, which help fulfill the mucosa's primary function: to protect the underlying tissues from external agents and fluid loss [11]. The drug to be designed must be able to cross the mucosal barrier. There are different factors to consider, such as the tissue's permeability, the drug's molecular weight, the partition coefficient (octanol/water) log P, and all aspects that are related to the formulation: the release capacity of the drug from the vehicle to tissue, pH, and the formulation's biocompatibility with the target tissue.

2. HPV Lesions and Other Issues in the Oral Cavity Treatment and Removal without Pain

We elaborated a 2% ketorolac tromethamine hydrogel composed of sodium alginate as a polymer to be applied to the buccal and sublingual mucosae with the aim of treating pain and inflammation before, during, and after surgical, ablative, or extractive procedures in the oral cavity. The hyaluronic acid was incorporated into the formulation because of its well-known regenerative, moisturizing and strengthening properties [12]. Both, low and high molecular weight hyaluronic acids have been used. The low-molecular-weight HA can penetrate to slightly deeper layers, and there, it can act regeneratively, while the high-molecular-weight HA acts at a more superficial level [13].

The physicochemical, mechanical, and morphological characteristics of the gel have been analyzed. The pH was within the normal intraoral pH range (6.8–7.8) [14], thus no disruptions, neither the biota nor the functions of saliva in the oral cavity, are expected. The alginate-HA gel showed to be hygroscopic in nature since it is able to uptake 15-fold its weigh in solvent, and their components can disperse in the medium relatively quickly compared to other polymer-based hydrogels. Mallandrich et al. studied the degradation of a 2% carbopol hydrogel, which required 24 h to be thoroughly degraded [15].

When formulating gels, determining the extensibility is crucial to ensure that the formulation is pleasant to use and has a comfortable application. That is why very high (very fluid) or very low (very viscous) extensibility should be avoided. The patient's compliance will be affected by the sensory feeling of the formulation. Inoue and co-workers investigated the correlation between the physical properties of different formulations and the sensory feeling [16]. This means that rheological studies are essential to evaluate the galenic features and the suitability of a formulation. Despite the compact structure of the alginate-HA 2% KT gel observed by SEM, the gel exhibited good extensibility and an ideal rheological behavior for the indication of the hydrogel. Pseudoplastic behavior is interesting because it allows a smooth and easy extension application by dabbing without high pressure and, therefore, painlessly. Furthermore, thixotropy also displays interesting behavior in semi-solid products because the formulation's change in structure results in fluidization that facilitates the product's application. This is an interesting outcome since the mucous membranes are already sensitive tissues per se and even more so after a surgical, ablative, or extractive intervention.

The biomimetic membrane PermeaPad® was tested and compared to the buccal and sublingual mucosae. It was observed that the biomimetic membrane correlated well with both mucosae. These results are in agreement with other researchers' work. Bibi et al. [17] investigated the use of PermeaPad® as a predictor in the buccal absorption of Metoprolol solution. The authors compared the apparent permeability obtained with PermeaPad® to the previous works performed by other authors, which evaluated the apparent permeability of metroprolol solution in cell culture, in ex porcine buccal mucosa and, finally, in in vivo studies conducted on minipigs. Bibi et al. found good in vitro–in vivo correlation between PermeaPad® and all the three systems evaluated.

Ketorolac tromethamine rapidly diffuses across the mucous membranes of the oral cavity, especially through the buccal mucosa. Under infinite doses and an exposure time of 6 h, ketorolac tromethamine would achieve therapeutic plasma concentrations. Nevertheless, the impact of saliva on drug elimination should not be disregarded, since the main drawback in buccal delivery is that the patient may swallow part of the applied dose before the drug is absorbed, even if it has been released [18]. The in vitro test showed that saliva dragged more than 60% of Ketorolac in 1 h, which is swallowed and follows on from an oral intake. Even despite the saliva's effect, Ketorolac tromethamine was able to penetrate both mucosae. The alginate-HA-hydrogel was formulated with Ketorolac in the tromethamine salt since this is more hydrophilic and allows it to be better integrated into the hydrogel. However, it is to be expected that once the gel is applied to the mucous membranes, the KT changes and, due to the environment in which it is found, protonates and/or ionizes and that, during the process, the different forms coexist until reaching a balance. These changes will affect the physicochemical properties of the drug, such as its solubility in saliva or the value of the log P partition coefficient, thus modifying its tissue affinity [19][20]. Given that the alginate-HA-hydrogel was formulated with the tromethamine salt, it is likely that this was the predominant form at the beginning of the study when applying the gel on the mucosa and therefore that the process of dissolving KT in saliva was favored. It should not be forgotten, however, that the KT of the formulation is simultaneously being absorbed through the mucosa. This absorption depends on different factors. On one hand, the mucosa histology, and on the other, the KT physicochemical properties. Taking into account that both the buccal and sublingual mucosae consist of a non-keratinized stratified squamous epithelium and that the main difference is the thickness of the said epithelium (the sublingual being between 100 and 200 µm, 8–12 cells thick, and between 500 and 800 µm, 40–50 cells thick, the buccal [21]), it is logical to think that the sublingual mucosa presents less resistance to the passage of KT. From the beginning, the amount of KT that is eliminated by the action of the saliva when the gel is applied on this mucosa is lower compared to when the gel is applied on the buccal mucosa. The kinetic dissolution profile is the result of the sum of these two processes. Through the buccal mucosa, it is observed how at the beginning, the KT dissolution in saliva

predominates until after about 30 min, when a balance is reached between what is dissolved and what is absorbed by the mucosa. This behavior adjusts to first-order kinetics. On the other hand, in the sublingual mucosa, by presenting less resistance to the passage of KT through its structure, the dissolution and absorption processes are balanced from the beginning, and this therefore describes zero-order dissolution kinetics. These results show how, apart from the drug physicochemical properties and the mucosa physiological characteristics, other factors such as salivation play a significant role in the bioavailability of KT since, in both mucosae, much more than half of the applied dose was eliminated by the action of saliva, which can be explained, in part, by the high-water solubility of KT, which is eliminated from the mucosa by the action of saliva and ends up being ingested. It is therefore considered that the KT is administered orally. In contrast, the amount that is retained in the mucosa is responsible for the local analgesic and anti-inflammatory action.

Thus, it is observed that the study in live animals and under a finite dose regimen does not reach systemic concentrations of KT. Besides the lower dose, this is probably due to the effect of saliva, as demonstrated in the in vitro study, which can influence by reducing the time that the alginate-HA gel is in contact with the mucous membranes and consequently with the amount of KT that could be permeated. It should be noted that the present study was carried out by administering a single drug dose in order to minimize the test time as much as possible and, therefore, the stress that could be caused to the animals. Other studies applying the alginate-HA gel in a multiple-dose regimen are necessary to analyze the behavior of the hydrogel more accurately in terms of bioavailability and permeability through the mucous membranes of the oral cavity.

When mucosae are damaged, their barrier functions are impaired, resulting in higher water loss [22]. This water loss can be measured by the transmucosal water loss (TMWL) method, which is well established in dermatology and used to assess the integrity of the mucosa barrier in vivo [23]. In the TMWL measurement, the water density gradient that evaporates through the tissue is indirectly measured by placing the measuring device perpendicular to the site of interest and reaching a stable TMWL reading in about 60 s. Before exposing the mucosa to alginate-HA hydrogel, the basal TMWL value was measured. The formulation was then applied to the mucosae, and after 2 h, the TMWL value was measured again. The TMWL values obtained, both basal and 2 h post-application, (around 30 $g/m^2 \cdot h$ for the buccal mucosa and around 40 $g/m^2 \cdot h$ for the sublingual mucosa) show the excellent condition of the mucosa since both have values close to 30 $g/m^2 \cdot h$, which is considered acceptable for the integrity of the oral mucosa [23]. Thus, the alginate-HA 2% KT does not cause mucosae disruption, being well-tolerated by the target tissues: the histological analysis revealed no differences between the treated and the untreated mucosae. Additionally, the cell viability showed that the alginate-HA 2% KT does not cause cytotoxicity in Caco-2 cells.

3. Conclusions

The objective of this study was to comprehensively characterize a hydrogel based on sodium alginate and high and low molecular weight hyaluronic acid formulated with 2% ketorolac tromethamine. Organoleptic, morphological, and rheological studies showed the suitability of the formulation to be an excellent and easy topical application of the hydrogel on the mucosa of the oral cavity.

The release studies demonstrated the remarkable capacity of KT to be released from the alginate-HA hydrogel, releasing 51.59% of the drug per cm^2 in 6 h.

Ex vivo permeation studies demonstrated good oral and sublingual mucosal patency for KT and predicted systemic steady-state concentrations within the therapeutic range. Furthermore, when comparing the results of both mucosae, no statistically significant differences were observed.

An additional positive finding was that comparative studies of the PermeaPad® biomimetic membrane with the buccal and sublingual mucosae showed an excellent correlation but a significantly lower drug retention capacity.

Through in vitro simulation, the influence of saliva on the bioavailability of the drug was observed. It was shown how in one hour, artificial saliva at a constant flow of 0.24 mL/min was capable of eliminating more than half of the initially applied dose on the mucous membranes, which would end up being swallowed and considered as oral administration.

In in vivo studies with pigs and under a finite regime dose, it was not possible to quantify the systemic concentrations of the drug, but the amounts of KT retained in both mucosae showed the feasibility of the gel to provide an analgesic and anti-inflammatory locally, which is very useful in surgical and/or ablative processes such as the elimination of papillomatous lesions, treatment of certain oral carcinomas, dental extractions, etc. Further studies are needed in a multi-dose regimen to characterize the hydrogel's behavior better, and this would be more realistic since, in practice, a single application of the formulation would not be sufficient to obtain the desired effect.

Finally, the histological, cytotoxicity study and the measured TMWL values demonstrated the safety and innocuousness of the formulation, not showing any damage or alterations to the mucosal tissues.

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